EFFECTS OF CENTRIFUGAL FORCE ON THE ECTOPLASMIC LAYER AND NUCLEI OF FERTILIZED SEA URCHIN EGGS

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In previous studies (E. B. Harvey, 1932, 1933*a*, 1933*b*) of sea urchin eggs stretched and broken apart by centrifugal force, it was found that unfertilized and fertilized eggs behave quite differently. The former form dumb-bells and pull apart as spheres of very definite size, while the latter during a certain period (monaster stage) form long streamers which break irregularly. A more extensive study has now been made of the fertilized eggs of some of the European sea urchins in which, owing to the clear, unpigmented protoplasm, the nuclear phenomena can be seen with great clearness in the living egg. It is possible by centrifugal force to separate the male and female pronuclei so that they come to lie either in different regions of an elongated egg, or in completely separated fragments. A study of the development of these eggs and fragments is presented in this paper, together with observations on the outermost layer of the fertilized egg (the ectoplasmic layer), which is actually peeled off the egg by centrifugal force. It has also been found that if eggs are centrifuged just before cleavage, the cleavage plane may come in without any relation to the new position of the mitotic figure.

Ectoplasmic Layer

The ectoplasmic or hyaline plasma layer of the sea urchin egg is a thin colorless layer appearing on the surface of the egg following fertilization, and is quite distinct from the fertilization membrane. It has an important function in holding the blastomeres together; the layer is disorganized and the blastomeres fall apart in the absence of calcium (Herbst, 1900; Gray, 1924, 1931; Moore, 1930). Some investigators (Ziegler, 1903; Gray, 1924, 1931; and Just, 1928) have considered it of importance in the mechanics of cell division, albeit for different rea-

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sons. The characteristic heaping of the layer at the equator at the beginning of cleavage would certainly lead one to believe that it is in some way concerned with the cleavage process.

In most sea urchins, the Naples species, *Paracentrotus lividus, Parechinus microtuberculatus, Sphærechinus granularis*, and the Woods Hole species, *Arbacia punctulata*, the layer, though detectable, is extremely thin (less than 1 μ) until ten or fifteen minutes after fertilization and it gradually becomes thicker until it measures 2 or 3 μ before cleavage (Fig. 1). In the Naples species of *Arbacia, A. pustulosa*, the ectoplasmic layer is well formed at the time that the fertilization membrane lifts off and even then measures 2–3 μ. It is particularly striking in this species as it makes a sharp contrast with the deeply colored egg. It is perfectly apparent here, by measuring the same egg (with a water immersion lens) before and after insemination, that the ectoplasmic layer is *added on* to the surface of the unfertilized egg. The diameter of the total egg increases 4–5 μ; this increase cannot be caused by any flattening of the egg due to gravity since the diameter of the colored portion remains the same. (See E. N. Harvey, 1933.) Glaser (1914, 1924) found (also by direct measurement) a *decrease* in diameter of the *Arbacia punctulata* egg on fertilization. In this species, however, it is to be noted that the ectoplasmic layer is extremely thin at this time so that any change in diameter due to it could scarcely be measured. His results have been questioned by Chambers (1921) and others. The layer seems to be, morphologically, in the nature of an extracellular membrane, rather than an integral part of the egg protoplasm, though this in no way detracts from its physiological importance. The behavior of the layer when subjected to centrifugal force leads to the same conclusion.

When the eggs of any of these sea urchins are centrifuged at any time after ten minutes following insemination, in a sucrose solution of the same density and tonicity as the eggs (in order to keep them suspended), at a centrifugal force of about 5000 $\times$ g, the ectoplasmic layer is centrifuged off the egg, usually in the form of a ring. It is best observed in eggs which have a large perivitelline space such as *Parechinus* (20 μ) and *Paracentrotus* (12 μ). When the eggs are first removed from the centrifuge, the ring is seen encircling the slightly tapering heavy pole of the egg, and the characteristic ectoplasmic layer investing the surface of the normal fertilized egg is lacking (Fig. 2, Photograph 1). As the egg becomes spherical, the ring slips off and lies free in the perivitelline space (Fig. 3). It remains here through cleavage and can be seen attached to the blastula (Fig. 4) even after it has become free-swimming, but eventually drops off. The ring is not always perfect, but is often incomplete or broken or has vacuolated or thinner

areas (Fig. 5). If eggs are centrifuged 7–10 minutes after fertilization, the ring is very narrow; if centrifuged before that, the ring does not form since there is an insufficient amount of ectoplasmic material. It may be centrifuged off also in the 2- and 4-cell stages, but is then of smaller size and more irregular shape (Fig. 6). The ring (and also the intact ectoplasmic layer) does not stain with any intravitam dyes; in such dyes as methylene blue, brilliant cresyl blue, and neutral red, it remains quite colorless, forming a sharp contrast to the heavily staining granular protoplasm. The ectoplasmic layer of the *Arbacia* egg (both *A. punctulata* and *A. pustulosa*) can also be thrown off, but owing to the small perivitelline space (1–5 μ), it is usually difficult to observe unless the fertilization membrane is broken at the centripetal pole and has slipped partly off, leaving a large space between it and the surface of the egg at the centrifugal pole. (Photograph 2.) In *Arbacia punctulata* it usually appears as a crescent rather than a ring. After the ectoplasmic layer from any of the eggs with intact fertilization membranes has been centrifuged off, it is gradually reformed on the surface of the egg, so that in about thirty minutes it looks almost the same as in uncentrifuged eggs, and after cleavage the blastomeres cohere. Perfectly normal plutei develop from these eggs in as large a percentage as from the controls. The ectoplasmic layer must also be replaced during the development of isolated blastomeres separated in calcium-free sea water, since, when returned to sea water, they may form perfect dwarf plutei whose cells cohere. It will be noted that the ectoplasmic layer differs markedly in this respect from the fertilization membrane, which is formed only once and is never replaced when once removed.

The ring-formation from the ectoplasmic layer, as described above, takes place in eggs centrifuged in an isotonic sucrose solution brought to the same density as the eggs by the addition of sea water; after centrifuging, the eggs were immediately returned to sea water. If the eggs

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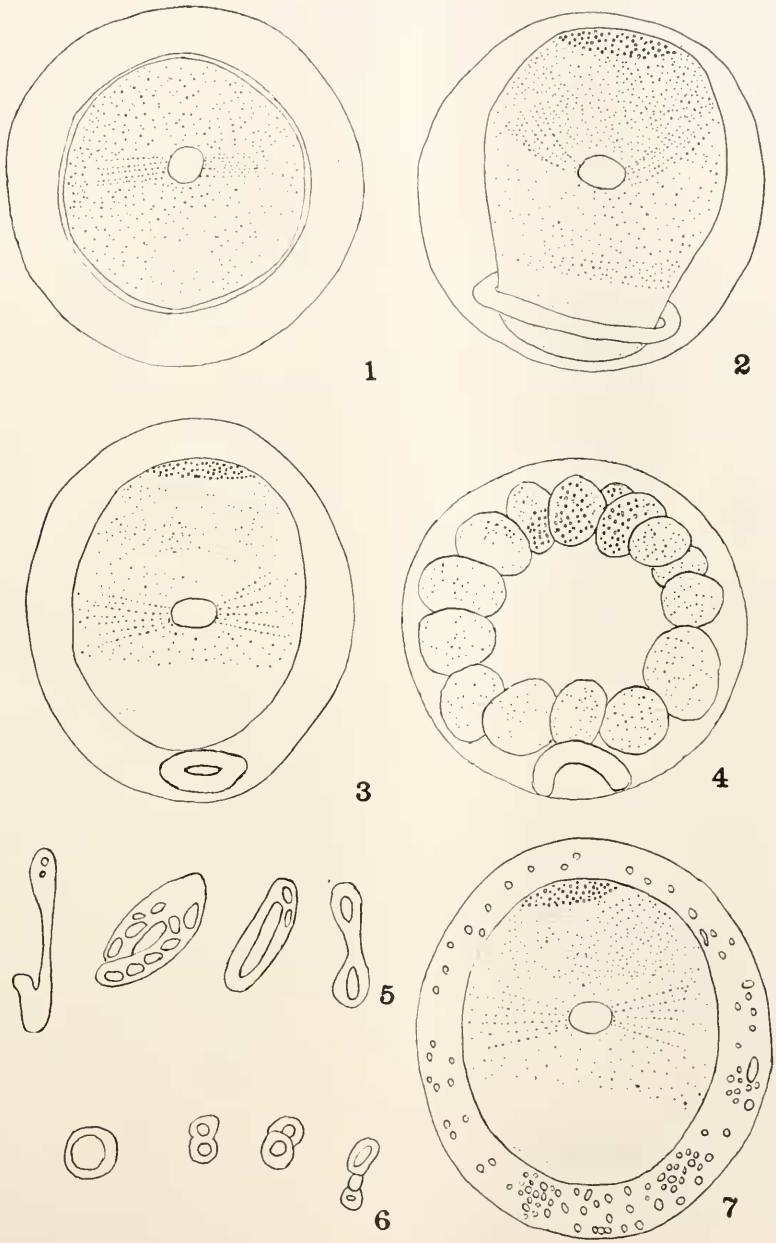
PLATE I

Parechinus microtuberculatus

1. Normal egg 30 minutes after insemination, showing ectoplasmic layer.
2. Egg centrifuged 30 minutes after insemination (for 3 minutes at 5,000 $\times$ g), showing ectoplasmic ring encircling heavy pole of elongate egg.
3. Same egg 10 minutes later; ectoplasmic ring lying in perivitelline space.
4. Same egg, early blastula; ectoplasmic ring still present.
5. Various forms of ectoplasmic ring when centrifuged off before first cleavage.
6. Various forms of ectoplasmic ring centrifuged off during 2-cell stage.
7. Egg kept and centrifuged in calcium-free medium, then put into sea water. Ectoplasmic material has precipitated in perivitelline space as small refringent bodies. Fertilized at 10:37; placed in calcium-free medium at 10:42; centrifuged from 10:47–10:51; then put in sea water; drawn at 10:55.

PLATE I



are put in *calcium-free* sea water five minutes after fertilization (*i.e.*, time to allow the fertilization membrane to harden), in order to disintegrate the ectoplasmic layer, and are then centrifuged in a mixture of this solution and isotonic sugar (same density as the eggs), the ring does not form. If the eggs after removal from the centrifuge are left in the same solution (*i.e.*, minus Ca) or transferred to calcium-free sea water, the perivitelline space remains perfectly clear and there is no ectoplasmic layer on the surface of the eggs. If the eggs, on removal from the centrifuge, are put in normal sea water, the perivitelline space becomes filled with many small refringent spherical or oval bodies (Fig. 7), the precipitation product of the ectoplasmic material in the presence of calcium. On return to calcium-free sea water, these are again dissolved and can be precipitated again in the presence of calcium. The ectoplasmic material, after being centrifuged off as a ring, may also be dissolved in calcium-free sea water and be precipitated again as scattered spherules when returned to sea water. Similar refringent spheres arising from the ectoplasmic layer have been described in eggs treated with acid sea water by Gray (1924, 1931) and by Moore (1928, 1932). The ectoplasmic layer, according to these two authors, reacts chemically like a calcium proteinate. Whatever its exact chemical nature, my experiments show that the ectoplasmic layer is a very definite external layer or membrane, highly unstable in the absence of calcium, easily peeled off by centrifugal force, and readily reformed under certain conditions after removal.

Protoplasm

The effects of centrifugal force on the protoplasm of the fertilized eggs of *Parechinus*, *Paracentrotus*, *Sphærechinus*, and *Arbacia pustu-*

Photographs 1–8

1. *Parechinus microtuberculatus.* Fertilized at 11:42; centrifuged from 11:52–11:58; taken at 11:59. Note ectoplasmic ring encircling heavy (small) pole of egg.

2. *Arbacia punctulata.* Centrifuged 8 minutes after fertilization. Ectoplasmic crescent within broken fertilization membrane.

3. *Parechinus microtuberculatus.* Fertilized at 10:15; centrifuged from 10:18–10:22 (5,000 × g). Eggs fragmented in many small pieces.

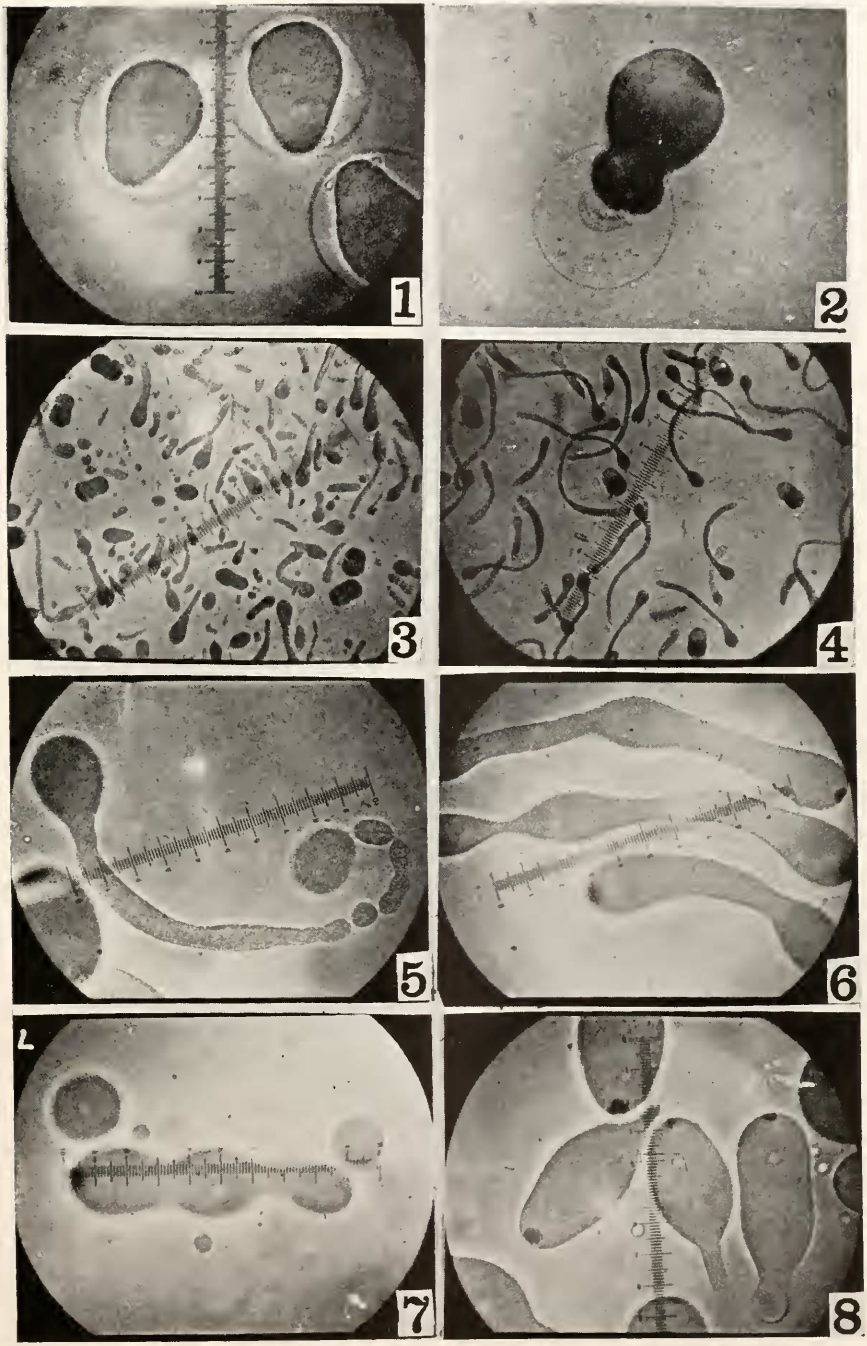
4. *Sphærechinus granularis.* Centrifuged 21 minutes after fertilization for 3 minutes (5,000 × g). Streamer stage, great elongation.

5. *Sphærechinus granularis.* Much elongated egg breaking up into small fragments.

6. *Parechinus microtuberculatus.* Fertilized at 8:50; centrifuged from 9:01–9:05. Taken at 9:15. Note bulge in egg where male aster is.

7. *Parechinus microtuberculatus.* Centrifuged 9 minutes after fertilization. Female nucleus below oil, ♂ nucleus in center; note bulge here.

8. *Parechinus microtuberculatus.* Fertilized at 10:15; centrifuged from 10:23–10:27. Egg more contracted; ♂ and ♀ nuclei present.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losa have been found to be practically the same as described for *Arbacia punctulata* (E. B. Harvey, 1933*b*). There is a period soon after fertilization (about 1½ to 6 minutes at 18° C.) when the eggs break very readily into many small pieces (Photograph 3); even slight shaking in the case of *Parechinus* and *Paracentrotus* at this period is sufficient to thoroughly fragment the eggs. The fertilization membrane at this time is elastic and easily broken; it sometimes stretches to twice its original diameter before breaking. After about six minutes it becomes quite tough and inelastic, and must be removed from the eggs (at 2 minutes after insemination by shaking) for experiments with the later stages. During or before the monaster stage (6–30 minutes after insemination, the time varying with the different species), the eggs elongate greatly, forming long streamers sometimes fourteen times the original diameter of the egg (Photographs 4–6); the streamers of the *Parechinus* and *Paracentrotus* egg are longer than those of the *Sphærechinus* egg. The streamers retract very quickly immediately upon removal from the centrifuge; a *Parechinus* egg which was stretched to 1300 μ retracted to 455 μ in five minutes. After the first few minutes they retract more gradually, often becoming spherical or nearly so, within an hour; they frequently break into small fragments, especially in the intermediate zone between the light and heavy poles which tend to remain somewhat intact (Photographs 4, 5). During the streak stage, the eggs elongate less and just before cleavage very little. The fertilized eggs of *Parechinus* and *Paracentrotus* break at all stages *more* readily than unfertilized eggs of the same batch centrifuged at the same time; the fertilized eggs of *Sphærechinus* break at all stages *less* readily than control unfertilized eggs. *Arbacia punctulata* is intermediate in this respect (E. B. Harvey, 1933*b*). In all the forms, the fertilized

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PLATE II

Parechinus microtuberculatus

8. Normal egg showing size of pronuclei at time of union; 14 minutes after insemination.

9. Egg fertilized at 9:45; centrifuged (5,000 × g) from 9:55–10:00; drawn at 10:10. Male nucleus measures 8 μ; ♀ nucleus 13 μ.

10. Same egg drawn at 10:25. Male and ♀ nuclei measure 13 μ.

11. Same egg drawn at 10:35. Male nucleus measures 19 μ; ♀ nucleus 8 μ. Note nucleolus in ♂ nucleus.

12. Same egg drawn at 10:40. Nuclei have fused and measure 20 μ.

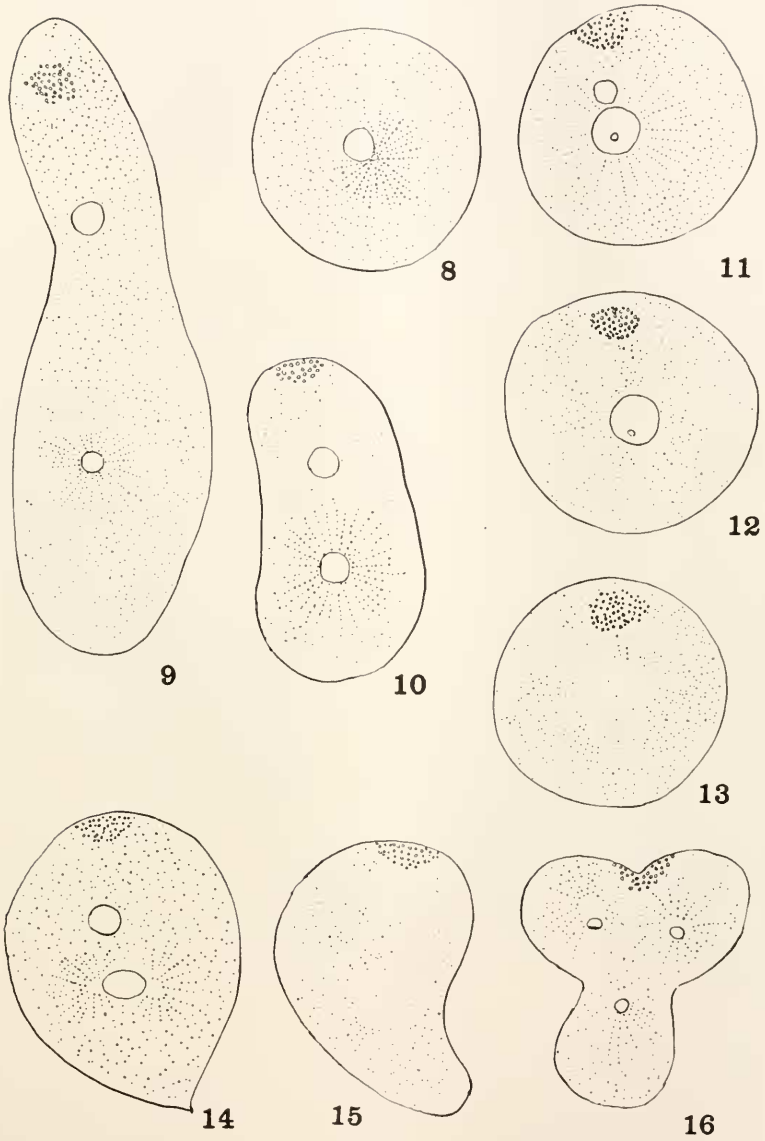
13. Same egg drawn at 11:45. Cleavage amphiaster.

14. Another egg. Fertilized at 11:28; centrifuged from 11:37–11:41; drawn at 12:07. Male nucleus forms amphiaster before union with ♀.

15. Another egg. Fertilized at 10:05; centrifuged from 10:12–10:16; drawn at 11:15. Male nucleus forms amphiaster, ♀ monaster.

16. Same egg at 11:22; tripolar cleavage.

PLATE II



eggs stratify less readily than the control unfertilized eggs, showing that they are more viscous; this difference is slight soon after fertilization but becomes more marked in later stages. As has been pointed out in the case of *Arbacia punctulata,* the greater ease of breaking must be due to surface differences between the fertilized and unfertilized eggs. The differences cannot, however, be due to the ectoplasmic layer, since this is scarcely formed at the time of maximal fragmentation and in later stages is thrown off by the centrifugal force as previously described.

Nuclei

In the eggs greatly elongated by centrifugal force (during or just before the monaster stage), the two pronuclei are thrown apart, the female pronucleus going to the light pole and the male to the heavy pole. The presence of the male nucleus in the heavy end of the eggs (of *Parechinus, Sphærechinus,* and *Paracentrotus*) is often made apparent first by the sperm aster. A pronounced bulging of the egg in this region (Photographs 6, 7) lends support to the generally accepted view that asters are of considerable rigidity and form regions of greater gelation. That the two nuclei should go to different poles is not surprising in view of the fact that at this time the male nucleus consists practically entirely of chromatin material in a very condensed form, whereas the female nucleus consists of the same material together with a large amount of other more fluid material. The spermatozoa are themselves heavier than the entire egg since they are thrown by centrifugal force to the bottom of a tube in the same medium in which the eggs remain suspended.

In normal uncentrifuged sea urchin eggs, the two pronuclei unite soon (6–15 minutes) after insemination while still very unequal in size (Fig. 8). By centrifuging the eggs, the two nuclei become so widely separated that it takes considerable time (an hour or more) for them to come together, which they do both because of the contraction of the egg protoplasm and by movement on their part through the protoplasm. During this time, the male nucleus increases enormously in size, so that it becomes not only of equal size with the female but often much larger; it frequently acquires a nucleolus while enlarging (Figs. 9–12, Photographs 7, 8). There is considerable variation in the actual size attained by the two nuclei; the female nucleus of *Parechinus* is about 13 μ in diameter, increasing to 16 μ before dissolving; the male nucleus has been observed to increase to 19 μ. The discrepancy in size is often much greater owing to the fact that the female nucleus may get smaller (before disappearing) at the same time that the nearby male nucleus is getting larger (Fig. 11). The fusion nucleus of the *Parechinus* egg usually measures 16 μ at its maximum, but has been observed to increase to 22 μ.

The male nucleus does not increase greatly in size (usually under 10 μ) when it is alone in a fragment obtained by centrifuging either before or after fertilization. This is probably due to the greater packing of granules in the surrounding protoplasm in these fragments as compared with the protoplasm surrounding the male nucleus when present (together with the female pronucleus) near the centripetal pole. The large size (22 μ) sometimes attained by the fusion nucleus in the clear quarter eggs of *Arbacia punctulata* where the surrounding medium is granule-free (E. B. Harvey, 1932) lends support to this explanation.

The importance of the time factor in controlling the size relations of the two nuclei is shown in the case of the eggs of many animals, annelids, mollusks, and even the closely related *Asterias* (Wilson and Mathews, 1895). The sperm here enters or may enter the egg before the polar bodies are given off and during the delay thus caused, the sperm nucleus increases in size until it is equal to the female. The two nuclei also become of equal size in the sea urchin *Toxopneustes,* if their union is delayed by treatment with ether (Wilson, 1902).

After the female and large male pronuclei unite as above described (Fig. 12), an amphiaster is formed and the egg cleaves normally (Fig. 13). Frequently the male centrosome divides before union of the two pronuclei, so that the advancing male nucleus is accompanied by an amphiaster instead of a monaster (Fig. 14). This is the case normally in *Asterias* and many annelids and mollusks and often occurs in etherized *Toxopneustes* eggs (Wilson, 1902). This amphiaster becomes or gives rise to the cleavage amphiaster after union of the two pronuclei, and normal cleavage follows. In some cases, the female nucleus decreases in size and disappears before union with the male, and the ensuing mitotic figure is then a triaster, the female nucleus being replaced by a monaster (Figs. 15, 16). The changes taking place in the male and female pronuclei and in the accompanying asters seem to be quite independent of each other and the variations occurring normally in different species can be duplicated in one species by varying the time relations of the events; this is accomplished in these elongate eggs produced by centrifugal force.

With a greater centrifugal force, the elongate egg can be broken into two parts, the lighter fragment containing the female nucleus and the denser fragment the male nucleus (Fig. 17). In the majority of experiments with both *Parechinus* and *Paracentrotus*, continuous observation of many of these half-eggs gave the following results. The fragment containing the female nucleus does not divide (Figs. 18–21). The nucleus enlarges (to about 16 μ), disappears, a large monaster

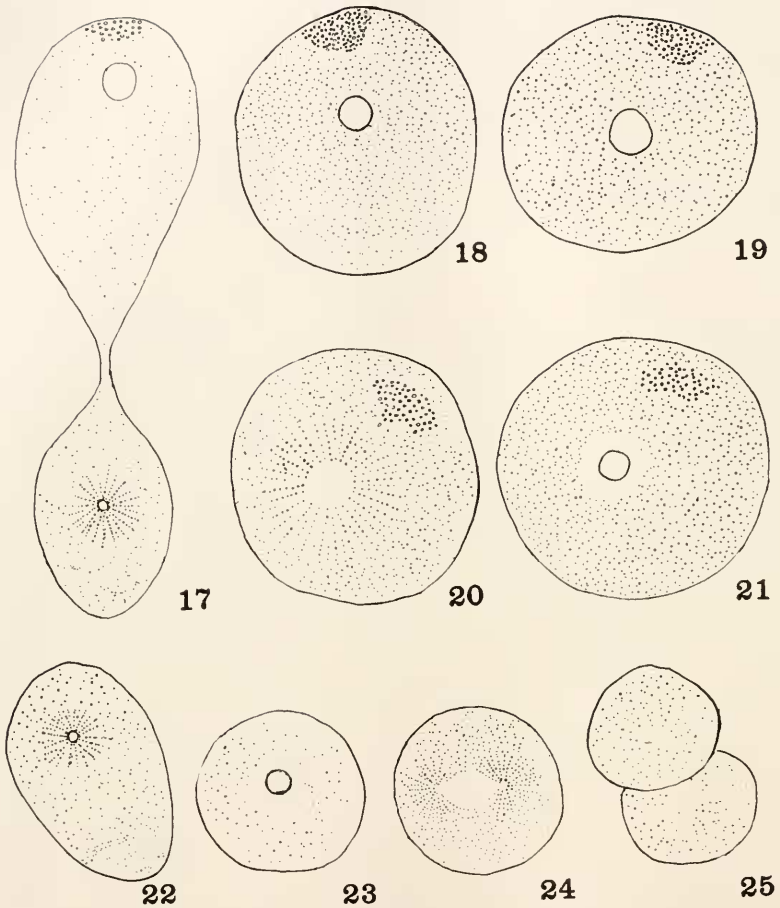


PLATE III

Parechinus microtuberculatus

17. Segregation of ♂ and ♀ nuclei into separate fragments; division center associated with ♂ nucleus. Fertilized at 10:05; centrifuged from 10:12–10:16; drawn at 10:26.

18–21. Development of fragment with ♀ nucleus.

18. Egg fertilized at 9:12; centrifuged from 9:15–9:19; fragment drawn at 9:35. Nucleus measures 13 μ.

19. Same fragment at 10:10. Nucleus measures 16 μ.

20. Same fragment at 10:45. Monaster.

21. Same fragment at 10:50. Nucleus back. Monaster again like Fig. 20 at 11:10; amœboid at 12:00.

22–25. Development of fragment with ♂ nucleus.

22. Fertilized at 10:05; centrifuged from 10:12–10:16; fragment drawn at 10:30. Sperm aster.

23. Same fragment at 10:40. Larger ♂ nucleus.

24. Same fragment at 11:15. Sperm amphiaster.

25. Same fragment at 11:30. Cleavage.

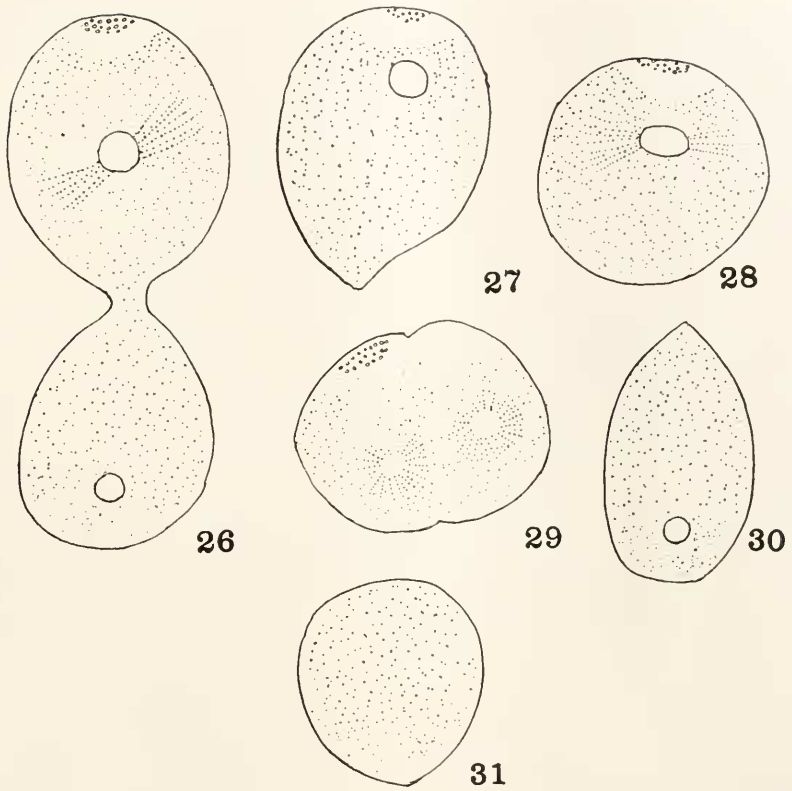


PLATE IV

Paracentrotus lividus

26. Segregation of ♂ and ♀ nuclei into separate fragments; division center associated with ♀ nucleus. Fertilized at 10:20; centrifuged from 10:37–10:41; drawn at 11:00.

27–29. Development of fragment with ♀ nucleus.

27. Egg fertilized at 12:08; centrifuged from 12:25–12:30; fragment drawn at 12:40. Nucleus enlarges to 16 μ.

28. Same fragment at 12:55. Rays from ♀ nucleus.

29. Same fragment at 1:20. Division.

30–31. History of corresponding fragment from same egg with ♂ nucleus.

30. Drawn at 12:40. No rays from ♂ nucleus.

31. Drawn at 12:55. Nucleus gone.

appears, the nucleus may later be reformed and again break down to form a monaster, often with a granular interior. This process may be repeated many times and finally the cell often becomes amœboid, but does not cleave. The fragment containing the male nucleus, on the

other hand, develops quite normally; the nucleus enlarges (to about 10 μ), then fades out and an amphiaster appears and the cell cleaves normally (Figs. 22–25). The active division center appears to be associated with the male nucleus; and the female nucleus, lacking it, is unable to form an amphiaster. This seems curious in view of the fact that the female nucleus can function alone and form an amphiaster in parthenogenesis. It was also found that in *Arbacia punctulata,* where the egg was separated inside the fertilization membrane, the white half containing the female nucleus alone frequently developed together with the red half containing the male nucleus alone, though this was not always the case (E. B. Harvey, 1933*b*). It is interesting to find that the eggs separated by hand with a cotton thread, so that one part contained the female nucleus and the other the male (in Ziegler's (1898) experiments with the same egg, *Parechinus*), behaved in exactly the same way as these separated by centrifugal force.

In two lots of *Paracentrotus* eggs, both from the same batch, centrifuged 17 minutes after insemination, the reverse of the usual occurrence took place in many eggs, though not in all. In the whole egg partially separated, the astral rays were associated with the female nucleus and none with the male (Fig. 26). When these eggs were completely separated, the amphiaster arose in connection with the female nucleus and this fragment divided (Figs. 27–29); no rays formed around the male nucleus, but it grew smaller and disappeared and the cell never divided (Figs. 30, 31). This same lot of eggs centrifuged earlier (15 minutes after insemination) gave the usual result, the amphiaster associated with the male nucleus; when centrifuged later (19 minutes) the two nuclei were not separated. It may be that there is a critical period of short duration when the active division center, arising,

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Photographs 9–15

9. *Sphærechinus granularis.* Centrifuged in spindle stage one hour after fertilization. Note oil drops in amphiaster in clear layer.

10. *Sphærechinus granularis.* Shows division plane through oil perpendicular to stratification.

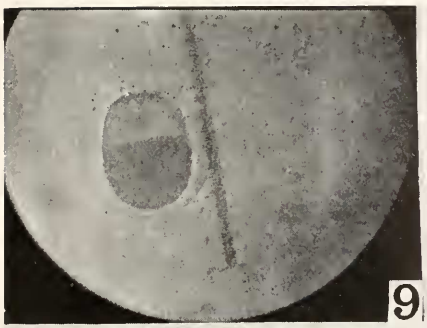
11. *Parechinus microtuberculatus.* Centrifuged just before cleavage. Note oil in astral rays.

12. Similar egg, showing division plane starting parallel with stratification and in no relation to mitotic figure, which is outlined by oil drops.

13. Similar eggs a little later. Note also disorganized ectoplasmic material in cleavage furrow.

14. *Paracentrotus lividus.* Centrifuged just before cleavage one hour after fertilization. Cleavage plane (in upper cell) has separated off clear cell which contains mitotic figure.

15. *Arbacia punctulata.* Centrifuged just before cleavage on centrifuge-microscope. Note central cell cleaved into one clear and one pigmented blastomere; the spindle is in the clear area.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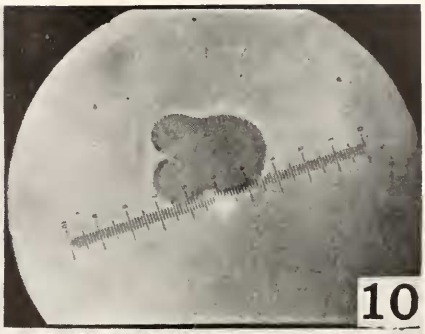
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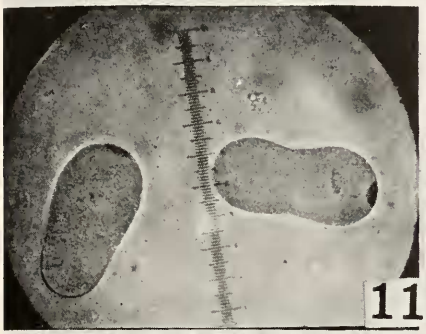
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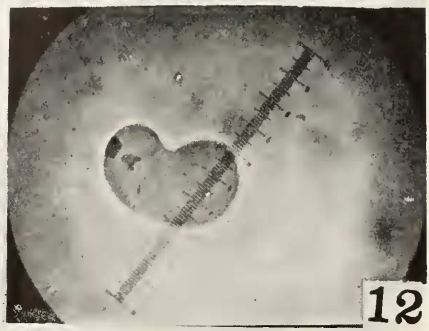
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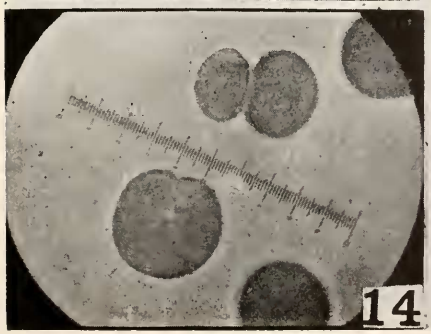
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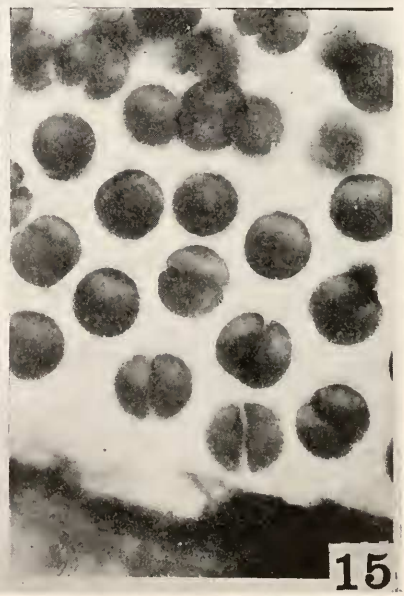
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as it usually does, in connection with the male nucleus, becomes independent of it or more closely associated with the female nucleus.

Amphiaster and Cleavage

When the eggs of any of the sea urchins are centrifuged after the amphiaster has formed, many of the oil drops are stopped in their passage to the light pole by the rays of the asters. In many cases the asters are completely outlined by the oil drops. This is particularly striking in eggs like *Sphaerechinus* and *Arbacia* where the mitotic figure lies in a perfectly clear area; this clear band is optically empty except for two small areas where the oil drops are enmeshed in the asters (Photograph 9). It is also quite striking even when the mitotic figure lies among granules as in the *Parechinus* egg (Photographs 11, 12). This demonstrates very clearly that the mitotic figure is a very definite structure consisting of more rigid or gelated material than the surrounding medium. The presence of the spindle and asters in the clear zone in fixed and stained material has been demonstrated by Spooner (1911) in the *Arbacia* egg.

If centrifuged for a sufficiently long time at a sufficiently high speed, the mitotic figure takes up its position under the oil cap with its long axis parallel with the stratification and the cleavage plane is perpendicular to the stratification (Photograph 10). This may occur even while eggs are rotating at fairly high speeds, as can be observed with the centrifuge-microscope (E. B. Harvey, 1933*b*). If, however, the eggs are centrifuged rapidly after the elongation preliminary to cleavage, the eggs orient with the long axis along the axis of centrifugal force (unless held in another position by the surfaces of the centrifuge-microscope slide). The spindle is still thrown into the light zone under the oil cap and oriented as before, but the cleavage plane may come in *parallel with* the stratification in the short axis and often in the exact position that it would have come in had the mitotic figure not been moved (Photographs 11–15). The cleavage plane thus bears no relation to the new position of the spindle, but to its former position. The change in the surface correlated with the initial location of the spindle is apparently sufficient for the formation of the cleavage plane in its original position. Whether chromosomes have been left behind and function in the formation of the cleavage plane has not been determined, as they cannot be seen in the living egg. The cleavage furrow comes in in this way, that is, without relation to the new position of the spindle, both in eggs while rotating, as can be seen with the centrifuge-microscope, and after the centrifugal force has been removed. The breaking strain due to centrifugal force also comes in this region, for at this time the eggs tend to break at or

near the equator into two fairly equal parts, whereas in earlier stages they break into unequal parts. A further investigation of the relation of the mitotic figure to the cleavage plane in the transparent eggs of *Parechinus* and *Paracentrotus* will be undertaken shortly, using the centrifuge-microscope.

Later Development of Eggs and Fragments

It has been noted above that when eggs are centrifuged with the fertilization membranes intact, they develop normally although the ectoplasmic layer is thrown off; this is apparently reformed if the eggs are protected by the fertilization membrane. They do not develop in any large percentage, however, if centrifuged during cleavage; this is no doubt owing to the disturbances of the mitotic figure (and chromosomes) mentioned above. It has also been noted previously that fragments without nuclei do not develop, and fragments containing only the female nucleus do not usually develop. Whole eggs and fragments other than those mentioned may develop and form swimming blastulae and plutei. In many cases, however, loose clusters of cells are formed owing to the lack of an ectoplasmic layer. This is true for eggs centrifuged soon after fertilization when the fertilization membranes are not properly formed or are destroyed, and for eggs from which membranes have been removed, and which have been centrifuged at any stage after fertilization. This applies to both whole eggs and fragments. No study has been made of the later development of individual eggs or egg fragments, but observation of experimental lots shows that failure to develop depends chiefly on the falling apart of the cells due to the lack of an ectoplasmic layer and that both large (from whole eggs) and small (from fragments) plutei occur in lots centrifuged at any stage after insemination.

Summary

1. The ectoplasmic layer is *added on* to the surface of the egg of *Arbacia pustulosa* on fertilization.

2. The ectoplasmic layer can be thrown off the fertilized eggs of *Parechinus microtuberculatus, Paracentrotus lividus, Sphærechinus granularis, Arbacia pustulosa,* and *A. punctulata* by centrifugal force as a ring or crescent which lies in the perivitelline space.

3. The ring is not formed in absence of calcium, but the dissolved ectoplasmic material is precipitated when the eggs are returned to sea water as refringent spherules in the perivitelline space.

4. The ectoplasmic layer is reformed on eggs with fertilization membranes and the eggs develop normally.

5. Soon after insemination (1½–6 minutes), all the species studied break into many very small pieces; during the monaster stage (6–30 minutes), they form long streamers; later, elongate dumb-bells. The fertilized eggs of *Parechinus* and *Paracentrotus* break more readily at all stages than unfertilized eggs, those of *Sphærechinus* less readily; the fertilized eggs of all species stratify less rapidly than the unfertilized.

6. The female pronucleus is driven by centrifugal force to the light pole and the male pronucleus to the heavy pole of the elongate eggs.

7. The male pronucleus may become much larger than the female before fusion; the size of the male nucleus depends on the time before union and the density of the surrounding protoplasm.

8. An egg may be broken into two fragments, one containing the female and the other the male nucleus. Usually the former forms a monaster and does not develop, the latter an amphiaster followed by normal cleavage. In two lots of *Paracentrotus* eggs, the reverse took place; the division center was associated with the female nucleus and this fragment divided while the other fragment with the male nucleus did not divide.

9. The spindle is thrown to the light pole and cleavage usually comes in through its equator, perpendicular to the stratification. If centrifuged just before cleavage, the cleavage plane may come in parallel with the stratification and in no relation to the new position of the spindle.

10. Many whole eggs centrifuged after fertilization develop normally and also many fragments thus obtained. Failure to develop is due to the lack of an ectoplasmic layer which causes the cells to fall apart, or to the absence of one or both nuclei.

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